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Molecular characterization of the progeny of *Solanum etuberosum* identifies a genomic region associated with resistance to potato leafroll virus

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Abstract Potato leafroll virus (PLRV; Genus *Polerovirus*; Family *Luteoviridae*) is one of the most important virus pathogens of potato worldwide and breeders are looking for new sources of resistance. *Solanum etuberosum* Lindl., a wild potato species native to Chile, was identified as having resistances to PLRV, potato virus Y, potato virus X, and green peach aphid. Barriers to sexual hybridization between *S. etuberosum* and cultivated potato were overcome through somatic hybridization. Resistance to PLRV has been identified in the BC_1, BC_2 and BC_3 progeny of the somatic hybrids of *S. etuberosum* (+) *S. tuberosum* haploid × *S. berthaultii* Hawkes. In this study, RFLP markers previously mapped in potato, tomato or populations derived from *S. palustre* (syn *S. brevidens*) × *S. etuberosum* and simple sequence repeat (SSR) markers developed from tomato and potato EST sequences were used to characterize *S. etuberosum* genomic regions associated with resistance to PLRV. The RFLP marker TG443 from tomato linkage group 4 was found to segregate with PLRV resistance. This chromosome region has not previously been associated with PLRV resistance and therefore suggests a unique source of resistance. Synteny groups of molecular markers were constructed using information from published genetic linkage maps of potato, tomato and *S. palustre* (syn. *S. brevidens*) × *S. etuberosum*. Analysis of synteny group transmission over generations confirmed the sequential loss of *S. etuberosum* chromosomes with each backcross to potato. Marker analyses provided evidence of recombination between the potato and *S. etuberosum* genomes and/or fragmentation of the *S. etuberosum* chromosomes.

Keywords Potato leafroll virus · *Solanum etuberosum* · *Solanum tuberosum*

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Introduction

Currently, none of the 13 most widely grown potato cultivars in North America are classified as having resistance to potato leafroll virus (PLRV; Genus *Polerovirus*; Family *Luteoviridae*) one of the most important virus pathogens of potato

worldwide (Corsini and Brown 2001). Planting of PLRV infected seed of a susceptible variety can result in yield losses of up to 80% (Bantarri et al. 1993). A worldwide estimate of yield losses attributable to PLRV annually is 20 million tons (Kojima and Lapierre 1988; Nolte et al. 2003). Losses were calculated as a composite of yield losses, downgrading and rejection of seed lots, and costs associated with the control of PLRV. Additional losses can be attributed to the reduced tuber quality of PLRV-infected tubers expressing net necrosis (Nolte et al. 2003). Breeding for resistance to PLRV in potato has been characterized as difficult due to a complex genetic control of resistance (Swiezynski et al. 1990; Barker et al. 1994; Jansky 2000), thereby explaining the lack of PLRV resistance in potato cultivars. However, there also have been a few reports of monogenic sources of resistance to PLRV (Barker and Solomon 1990; Brown and Thomas 1994).

New sources of PLRV resistance from the wild relatives of potato, especially if monogenic or oligogenic in expression and inheritance, would be of benefit to potato breeders in developing PLRV resistant potato cultivars. *Solanum etuberosum* Lindl., a wild potato species native to Chile, was shown to have resistances to PLRV, potato virus Y (PVY), potato virus X (PVX), and green peach and potato aphid (USDA ARS National Genetic Resources Program 2003). Classified as a 1 EBN species, *S. etuberosum*, has been characterized as having an E-genome distinct from the A-genome of *S. tuberosum* (Matsubayashi 1991). With respect to taxonomy, *S. etuberosum* is classified in *Solanum* sect. *Etuberosum*, distinct from the tuber-bearing *Solanum* species in sect. *Petota* (Spooner and Hijmans 2001). The distinct genomic and taxonomical differences between *S. etuberosum* and cultivated potato have made sexual hybridization difficult. Barriers to sexual hybridization were overcome through the use of somatic hybridization (Novy and Helgeson 1994; Thieme et al. 1999).

Resistance to PLRV in species of sect. *Etuberosum*, has been characterized by little or no accumulation of virus and simple genetic control (Chavez et al. 1988; Valkonen et al. 1992). This was confirmed by the identification of reduced PLRV accumulation in BC_1, BC_2 and BC_3 progeny of the somatic hybrids of *S. etuberosum* (+) *S. tuberosum* haploid × *S. berthaultii* Hawkes following the grafting of PLRV-infected scions (Novy and Gillen, unpublished data). BC_3 clones have been identified in our program with PLRV titers similar to the resistant BC_2 parent indicating PLRV resistance from *S. etuberosum* is highly heritable. Multi-year field nurseries in our program have shown that these BC_1, BC_2 and BC_3 progeny have high levels of field resistance as measured by ELISA testing of progeny tuber plants (Novy and Gillen, unpublished data). In addition, all BC_2 derived from *S. etuberosum* express some level of resistance to aphid, a known vector of PLRV (Novy et al. 2002).

A very important issue for the successful incorporation of resistances from *S. etuberosum* into cultivated potato with minimal linkage drag of undesirable genes from the wild parent is the amount of recombination between the A- and the E-genomes. Lack of homology between chromosomes results in reduced pairing during meiosis and hence reduced recombination. The E-genome map from a *S. palustre* (syn *S. brevidens*) × *S. etuberosum* cross (Perez et al. 1999) provided evidence of significant colinearity among A and E chromosomes and significant translocations and inversions which differentiate the two genomes. Perez et al. (1999) states "Definitely groups 2, 8, 9 and 10 and possibly groups 1, 4 and 12 in the E-genome are structurally different from their homologues in the A-genome." However, this E-genome map consisted of 19 linkage groups (LGs), which leaves some uncertainties in interpreting the structural differences between the A- and E-genomes.

Previous work on populations derived from backcrossing *S. tuberosum* (+) *S. palustre* (syn. *S. brevidens*) somatic hybrids to *S. tuberosum* showed that RFLP markers used in tomato and potato mapping (Bonierbale et al. 1988; Tanksley et al. 1992) could be used to follow the E-genome chromosomes through generations (Williams et al. 1990; McGrath et al. 1996). They produced BC_2 individuals with varying numbers of presumably unpaired E-genome chromosomes. These clones were analyzed with RFLP markers to identify retained E-genome regions and to investigate the recombination between the A- and E-genomes.

Marker synteny, i.e. markers that are found together and *may* be located on the same chromosome, not marker order, can be inferred from this (McGrath et al. 1994, 1996) experimental design.

In this analysis, instead of linkage groups we refer to 'synteny groups'. McGrath et al. (1994) analyzed 17 BC_2 progeny from six fertile BC_1 plants. They assumed their markers had the order of the potato linkage map (Tanksley et al. 1992) because they did not have Perez's E-genome map (Perez et al. 1999) to work with. Their findings provide evidence of synteny among groups of markers in the E-genome in that markers localized to chromosomes 1,2,5,6,11 and 12 in potato and tomato were lost as a group in some BC_1 individuals, i.e. if one marker from chromosome 1 was missing than the other chromosome 1 markers were missing. Conversely, if only a portion of the markers in a synteny group are present in the offspring, then this is evidence for either recombination or a lack of synteny between the E-genome and A-genome chromosomes. In other words, recombination and segregation cannot be differentiated. This line of analysis was used in our studies because our populations are not large enough for linkage analysis to be meaningful.

This research was conducted using molecular markers to localize genomic regions of *S. etuberosum* which are associated with PLRV resistance in segregating BC_2 and BC_3 populations. Localization of genomic regions conferring PLRV resistance would be useful in allowing further saturation of those regions with additional molecular markers closely linked to these resistance genes. This will be useful to facilitate introgression into cultivated potato of these genes while retaining the minimum amount of *S. etuberosum* genome.

An additional component of our research was an evaluation and comparison of synteny groups in our *S. etuberosum*-derived population with previously published potato, tomato, and *S. palustre* (syn *S. brevidens*) × *S. etuberosum* maps. Such comparisons also allowed an assessment of the potential for recombination between the A- and E-genomes—an important consideration in the introgression of resistances from *S. etuberosum* to cultivated potato.

Materials and methods

Plant materials

Protoplast fusion of *S. etuberosum* (PI 245939) with a haploid-species hybrid, 463-4 [*S. tuberosum* subsp. *tuberosum* haploid (US-W 730) × *S. berthaultii* (PI 265857)] produced regenerant clones. Chromosome counts and analyses of regenerants with RFLP, isozyme, and GISH confirmed their hybridity (Novy and Helgeson 1994; Dong et al. 1999). Background for the introgression of PLRV resistance from *S. etuberosum*, as well as details of the plant material used in this study are presented in Fig. 1 and Table 1. The specific *S. etuberosum* clone used was designated "16-1" and was maintained via tissue culture because it does not produce tubers. Potato cultivars 'Katahdin' and 'Atlantic' were crossed with the somatic hybrids to produce the BC_1 and BC_2. PLRV resistant BC_1 clones P2-3 and P2-4, and six BC_2 progeny of P2-3 (Etb 5-31-2, Etb 5-31-3, Etb 5-31-4, Etb 6-21-3, Etb 6-21-5 and Etb 6-21-12) were utilized in this study (Table 1). Four years of field trials in Idaho have shown Etb 6-21-3 and Etb 5-31-2 to have high levels of PLRV field resistance, with less than 10% infected daughter tubers relative to susceptible Russet Burbank with 98% infected daughter tubers in the same trials (Novy, unpublished data). The remaining BC_2 clones are

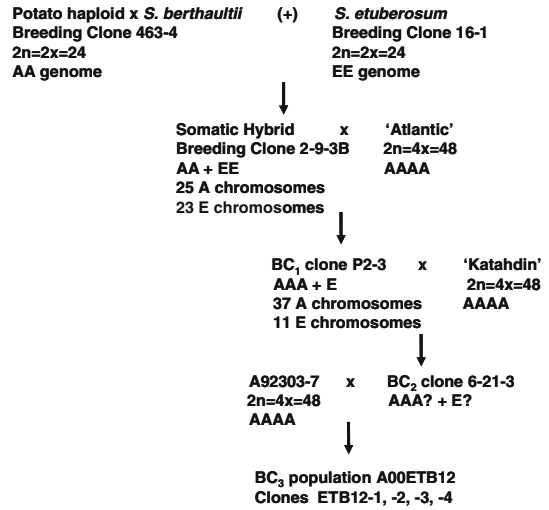


Fig. 1 Diagram illustrating the sequential introgression of PLRV resistance from *S. etuberosum* into cultivated potato

Table 1 Description of clones

Entry	Description	Parentage	PLRV Response[a]
16-1	*S. etuberosum* Fusion parent	Seedling from PI 245939	R[b]
463-4	Fusion parent of somatic hybrid	US-W730 × *S. berthaultii*	S
2-9-3B	Tetraploid somatic hybrid	463-4 + 16-1 (*S. etuberosum*)	R
Atlantic	Parent of BC_1 clone, P2-3	Wauseon × Lenape	MS
P2-3	BC_1 of somatic hybrid	Somatic hybrid × Atlantic	R
P2-4	BC_1 of somatic hybrid	Somatic hybrid × Katahdin	R
Katahdin	Parent of P2-4 & BC_2	USDA 40568 × USDA 24642	MS
Etb 5-31-2	BC_2 of somatic hybrid	P2-3 × Katahdin	R
Etb 5-31-3	BC_2 of somatic hybrid	P2-3 × Katahdin	MS
Etb 5-31-4	BC_2 of somatic hybrid	P2-3 × Katahdin	S
Etb 6-21-3	BC_2 of somatic hybrid	P2-3 × Katahdin	R
Etb 6-21-5	BC_2 of somatic hybrid	P2-3 × Katahdin	MS
Etb 6-21-12	BC_2 of somatic hybrid	P2-3 × Katahdin	S
A86102-6	Parent of BC_3	A7532-1 × A8173-4	S
A92303-7	Parent of BC_3	A86332-7 × Ranger Russet	S
A95109-1	Parent of BC_3	Blazer Russet × Summit Russet	S
A00ETB12-1	BC_3 of somatic hybrid	A92303-7 × Etb 6-21-3	S
A00ETB12-2	BC_3 of somatic hybrid	A92303-7 × Etb 6-21-3	R
A00ETB12-3	BC_3 of somatic hybrid	A92303-7 × Etb 6-21-3	R
A00ETB12-4	BC_3 of somatic hybrid	A92303-7 × Etb 6-21-3	S
A00ETB11-1	BC_3 of somatic hybrid	A86102-6 × Etb 6-21-3	S
A00ETB14-1	BC_3 of somatic hybrid	A95109-1 × Etb 6-21-3	S
A00ETB21-7	BC_3 of somatic hybrid	Etb 6-21-5 × A86102-6	S
A00ETB21-12	BC_3 of somatic hybrid	Etb 6-21-5 × A86102-6	S

PLRV reaction was determined based on multiple years of field evaluations in Idaho and Minnesota and from grafting studies

[a] R = Resistance, MS = Moderate Susceptibility, and S = Susceptible

[b] PLRV response was made on the basis of GRIN Data concerning PI 245939 which indicated it had PLRV resistance, literature concerning the PLRV resistance of *S. etuberosum*, and the high level of resistance found in the BC progeny which was not evident in the other fusion parent, 463-4

considered susceptible to PLRV infection with statistically higher percentages of infected daughter tubers relative to resistant Etb 6-21-3 and Etb 5-31-2.

The BC_3 family designated A00ETB12 (A92303-7 × Etb 6-21-3) consists of two PLRV resistant clones abbreviated as ETB12-2 and ETB12-3, and two PLRV susceptible clones ETB12-1 and ETB12-4. Three years of field evaluation have shown ETB12-2 and ETB12-3 to be as resistant to PLRV as their BC_2 parent, Etb 6-21-3 (data not shown). Few BC_3 plants were produced per cross in initial hybridizations between potato and the PLRV resistant BC_2 individual 6-21-3. Therefore, PLRV susceptible BC_3 clones ETB11-1 (A86102-6 × Etb 6-21-3), ETB14-1(A95109-1 × Etb 6-21-3), ETB0021-7 (Etb 6-21-5 × A86102-6) and ETB0021-12 (Etb 6-21-5 × A86102-6) and their potato parents A86102-6 and A95109-1, were included as susceptible controls. The designation of these additional BC_3 clones and their parents as PLRV susceptible was based on 1–2 years of field evaluation.

Protocols used in screening for PLRV resistance

Plots of entries consisted of five hills replicated three times in a randomized complete block (RCB) design. Field testing consisted of the use of PLRV infected spreader rows interspersed among entry rows. Spreader rows provided virus inoculum for dispersion by native aphid populations. Details of this field screening protocol are described in Corsini et al. (1994).

Tubers from the trial were harvested, and 10 tubers from each plot were planted in the greenhouse. Emerged daughter plants were then assayed for PLRV using DAS-ELISA. PLRV antibodies were obtained from BioReba, Ag®, Reinach, Switzerland. Daughter plants with

absorbance values of ≥0.1 were classified as infected with PLRV. Percentages of infected daughter tubers for each entry in each replication were obtained. Statistical analyses were conducted using JMP® Software (SAS, Cary, North Carolina).

As indicated in the field screening protocols, native populations of aphids were used to vector PLRV. BC_2 clones used in this study were previously shown to have resistance to green peach aphid (Novy et al. 2002). However, it is unlikely that aphid resistance confounded PLRV resistance ratings, since high levels of PLRV infection were observed in the daughter tubers of aphid resistant BC_2 clones having no genetic resistance to PLRV (Novy et al. 2002).

DNA extraction and RFLP analysis

Young leaf tissue of plants grown in the field or growth chamber was used for DNA extraction using either a CTAB extraction as described by Doyle and Doyle (1987) or a nuclei extraction procedure as described in Bernatsky and Tanksley (1986) except that 0.02 M Na-Bisulfite was used instead of B-mercaptoethanol, chloroform/isoamyl alcohol instead of chloroform/octanol and the purified DNA was treated with Rnase A instead of CsCl/ethidium bromide centrifugation. DNA from each clone was digested with *EcoRI*, *EcoRV*, *DraI*, *HindIII* or *XbaI*, precipitated, dried, rehydrated and then 10 ug of the digested DNA was loaded and separated on a 0.8% 1× TAE agarose (SeaKem® LE, FMC) gel. Southern blotting was performed using the alkaline transfer protocol (Sambrook et al. 1989) and Hybond N+ membrane (Amersham Pharmacia Biotech, Piscataway, NJ).

A total of 59 tomato genomic and cDNA clones were used as probes (Bonierbale et al. 1988). These probes were selected because they were shown to be polymorphic in populations derived from Solanum section *Etuberosum* species [*S. etuberosum* and *S. palustre* (syn: *S. brevidens*)] and *S. tuberosum* (Williams et al. 1990; Novy and Helgeson 1994; McGrath et al. 1996) and/or were used for genetic linkage mapping of the E-genome (Perez et al. 1999). Only markers unique to the *S. etuberosum* parent (E-genome) were analyzed. Probes were obtained either as plasmids or PCR products from Dr. Steven Tanksley, Cornell University; Dr. John Helgeson, University of Wisconsin (retired); or Dr. Roger Chetelet, Tomato Genetics Resource Center, University of California, Davis, CA. RFLP probes were amplified using PCR (M13F and M13R primers) and labeled using the Gene Images™ Random Prime Labeling and Detection System (Amersham Pharmacia Biotech). Hybridization and detection were carried out using the Gene Images CDP-Star detection module (Amersham Pharmacia Biotech).

Optimization of SSR markers

SSR markers developed from potato (Milbourne et al. 1998) and tomato sequences (Van der Hoeven et al. 2001) published online by the Solanaceae Genomics Network (2003) that had been mapped in tomato or potato (http://sgn.cornell.edu) (Van der Hoeven et al. 2001) were chosen. Only SSR markers that mapped in potato or tomato to linkage groups 3, 4, 6 and 11 were used. Markers specific to these chromosome regions were selected based on prior RFLP analysis which identified them as potentially being associated PLRV resistances (LGs 3, 4, and 6) and chromosome 11 markers were included as a check because a major gene for quantitative PLRV resistance in potato was identified on chromosome 11 (Marczewski et al. 2001). SSR markers SSR22, SSR31, SSR46, SSR47, SSR67, SSR72, SSR76, SSR80, SSR111, SSR128, SSR136, SSR146, SSR188, SSR231, SSR293, SSR300, SSR310, SSR340, SSR350, SSR578, STM0001, STM0019, STM0025, STM0037, STM1025, STM1058, STM1069, STM1100, STM2005, STM3016, and STM3020 from Milbourne et al. (1998) and Van der Hoeven (2001) were tested.

PCR reaction and thermal cycle conditions were modified from Milbourne et al. (1998). Optimized PCR reaction conditions were 1× PCR buffer (Sigma)[10 mM Tris–HCl, pH 8.3 at 25°C, 50 mM KCl, 1.5 mM $MgCl_2$ and 0.001% gelatin], 1.0 mM $MgCl_2$ (total in reaction is 2.5 mM), 0.2 mM each dNTP, 0.25 U (total amount) Taq DNA polymerase (Sigma), 7.5 pM of forward primer, 7.5 pM of reverse primer, 20 ng of

template in 10 µl total volume. PCR reaction conditions were 94°C for 3 min, anneal at (58–64°C) for 2 min, 72°C for 1.5 min—one cycle; 94°C for 30 s, anneal (58–64°C) for 1 min, 72°C for 30 s—38 cycles; 72°C for 20 min, soak at 5°C. Annealing temperature was optimized for each SSR using the gradient feature of the MJ Research DNA Engine thermal cycler. Initially, PCR products were visualized on Seakem LE Agarose 0.7%/Synergel 1.65% gel. After optimization, forward primers labeled with fluorescent dye IR700 were used and the PCR products were visualized on 6.5% polyacrylamide gel (Genotyping KB plus 6.5% gel matrix with urea and TBE) using either the LI-COR IR^2 3200 DNA sequencer or LI-COR 3300 DNA analyzer.

Synteny group construction

The expected order of the RFLP and SSR markers in each synteny group (Table 2 and Fig. 2) was based on marker order from the E-genome map of Perez et al. (1999), the potato linkage map (Tanksley et al. 1992) and the Tomato EXPEN1992 and Tomato EXPEN2000 linkage maps (Bonierbale et al. 1988; Van der Hoeven et al. 2001). SSR markers had been mapped in the *S. lycopersicum* (previously known as *Lycoperiscon esculentum*) LA925 × S. pennellii "EXPEN2000" F_2 population (Van der Hoeven et al. 2001) which also contains a subset of the RFLP probes used to map *S. lycopersicum* cv. VF36 × *S. pennellii* LA716 type F_2, "Tomato-EXPEN 1992" from Tanksley et al. (1992), therefore the SSR markers could be related to the potato map, though they have not been mapped directly in potato. Only markers which were unique to the *S. etuberosum* parent were analyzed.

Results

RFLPs

The *S. etuberosum*-derived BC_2 population and its parental clones and P2-4 were screened for restriction fragment length polymorphisms (RFLP) using 59 tomato probes expected to cover all linkage groups in the A- and E-genomes.

Fifty-three of the 59 RFLP probes detected 54 bands unique to *S. etuberosum* and six probes were either monomorphic or gave ambiguous results. All informative RFLPs previously associated with synteny groups 3, 4, 6 and 11, and selected RFLPs from the other synteny groups (41 markers) were screened against the BC_1 parent P2-3, the segregating BC_3 family ETB12 and its parents, the PLRV resistant BC_1 clone, P2-4, and the PLRV susceptible control individuals ETB11-1, ETB14-1, ETB21-7, ETB21-12, A95109-1 and A86102-6. Two of the forty-one probes were not polymorphic among the BC_3 parents and one probe did not produce a clear signal.

Analyses of the BC_2 identified 3-7 RFLP markers per synteny group. Markers from all 12 synteny groups were represented in the BC_1 clones. Markers from all synteny groups were passed to at least one clone in the BC_2 and each clone had 5–8 synteny groups (Table 3).

RFLP probes TG123 and TG208, both from synteny group 4, and each detected two distinct bands that are unique to 16-1, which most RFLPs did not do. In each case, the two bands detected by a single probe and enzyme combination segregated independently in the next generation. RFLP marker CD65 mapped to linkage groups 6 and 7 in the E-genome map and produced two segregating RFLP bands unique to 16-1. Probe TG22, also from synteny group 4, detected a faint band in ETB12-1, no band in ETB12-4, and a very strong band in ETB12-2 and ETB12-3. This could indicate that resistant clones have two copies of this region and the susceptible have one or none.

SSRs

Thirty-one SSRs targeting linkage groups 3, 4, 6 and 11 were used. Twenty SSR markers from Van der Hoeven et al. (2001) gave 18 clear PCR products of which 11 SSR products were polymorphic, unique to *S. etuberosum* and scorable in the BC_2 (Table 2). None of these SSRs produced a band in the *S. etuberosum* parent that was not present in the somatic hybrid. The Milbourne et al. (1998) SSRs were not used in the analysis because those primers that produced a product in *S. etuberosum* gave inconsistent results or did not produce a scorable PCR product in the BC_2. Four Milbourne

Table 2 Summary of markers used to construct E-genome synteny groups

Probe/marker	Expected synteny group	Expected synteny group order	Linkage group in E-genome map	Potato/tomato linkage group
CD2	12 or 2	7	2	12
CD8	9	2	9	9
CD19	12	4	n.a.	12
CD35	2	4	n.a.	2
CD64	5	1	n.a.	5
CD65	6 and 7	1	6 and 7	7
CD67	6	2	6	6
CD65[a]	6 and 7	1?	7 and 6	7
CT148	8	2	8	8
CT182	11	5	11	11
SSR22	3	3	n.a.	3
SSR31	3	5	n.a.	3
SSR67	11	4	n.a.	11
SSR76	11	6	n.a.	11
SSR111	3	2	n.a.	3
SSR128	6	4	n.a.	6
SSR136	11	1	n.a.	11
SSR146	4	5	n.a.	4
SSR188	4	8	n.a.	4
SSR310	4	2	n.a.	4
SSR350	6	7	n.a.	6
TG8	9	4	n.a.	9
TG17	1	3	1	1
TG18	9	1	n.a.	9
TG22	4	7	n.a.	4 and 3
TG26	11	9	n.a.	11
TG31	2	1	n.a.	2
TG34	2	5	n.a.	2
TG44	11	7	n.a.	11
TG46	11	8	11	11
TG63	10	3	n.a.	10
TG65	4	4	n.a.	4
TG68	12	3	12	12
TG115	6	6	6	6
TG122	10	2	n.a.	10
TG123	4	1	4	4
TG125	1	2	1	1
TG128	7	4	n.a.	7
TG135	3	1	3	3
TG143	7	5	7	7
TG180	12	1	12	12
TG185	5	3	n.a.	5
TG194	11	2	n.a.	11
TG208	4 or 1	3	1	4
TG230	10	1	10	10
TG240	6	3	6	6
TG244	3	6	n.a.	3
TG261	8	1	8	8
TG275	6	5	n.a.	6
TG276	2	2	2	2
TG296	12	5	n.a.	12
TG301	1	1	n.a.	1
TG360	12	2	n.a.	12
TG377	3	4	3	3
TG379	5	2	5	5
TG390	9	3	9	9

Table 2 continued

Probe/marker	Expected synteny group	Expected synteny group order	Linkage group in E-genome map	Potato/tomato linkage group
TG393	11	10	11	11
TG402	8	3	n.a.	8
TG408	10	4	n.a.	10
TG438	7	2?	n.a.	7
TG443	4	6	4	4
TG462	2	3	2	2
TG468	12	6	12	12
TG508	11	3	11	11
TG572	7	3	7	7

E-genome map order is from Perez et al. 1999. Linkage data in tomato and potato from the Solanaceae Genomics Network database at http://sgn.cornell.edu was also used to construct synteny groups. The RFLP probes are designated TGxxx, CDxxx or CTxxx (Tanksley et al. 1992; Van der Hoeven et al. 2001). The SSR are designated SSRxxx (Van der Hoeven et al. 2001). Linkage data for potato and tomato was last updated on 4/14/05 by accessing the linkage maps at http://sgn.cornell.edu

[a] CD65 produced two unique bands in the *S. etuberosum* parent. One was 5 kb and had the same distribution in the BC_2 population as markers from synteny group 7 and the other was 7.9 kb and fit the pattern of synteny group 6 markers

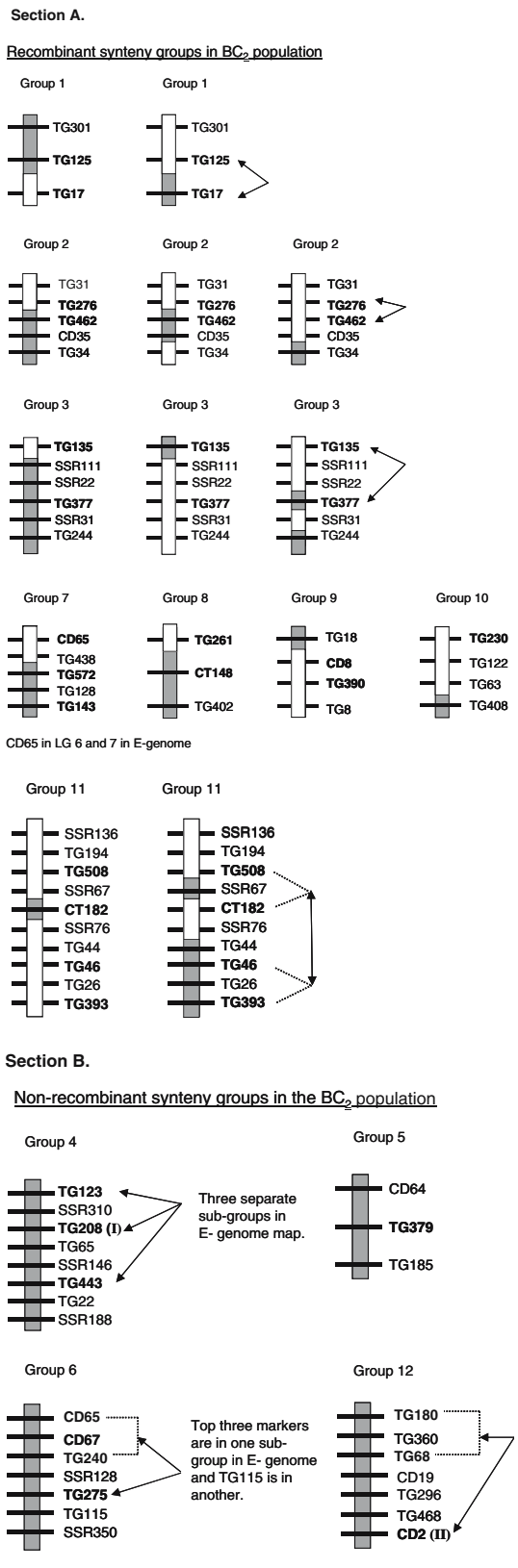
SSR primer sets produced a unique PCR product from *S. etuberosum* but three of these were not present in any BC_2 [STM0019 (LG 6), STM1025 (LG 3), STM3016 (LG4)] and the 4th one was unreliable when repeated in the BC_2. Also, four Milbourne SSRs produced products in the parents of the somatic hybrid, yet they were not present in the somatic hybrid itself. This was contradictory to the RFLP data.

Integration of the SSRs created some unexpected results. Clone 6-21-12 did not have the three SSRs from synteny group 3 despite the presence of two of the three RFLPs from this group. This created a triple recombinant synteny group 3, which is highly unlikely (Fig. 2). Also, SSR67 in synteny group 11 created a triple recombinant synteny group in one clone. SSR67 produced two products in the 16-1 parent (Table 4) but only the 61 bp band was polymorphic. In tomato SSR67 also produced two products but was only mapped to linkage group 11. These may indicate that our assumed marker order in synteny group 11 may be incorrect or the 61 bp band from *S. etuberosum* is not from a region that is homologous to potato/tomato linkage group 11.

The order of markers in the E-genome linkage groups 4 and 6 corresponded with the potato order except for some questionable duplicated loci. In determining the similarity or difference between two linkage maps duplicated loci are problematic because different bands detected by the probe could have been scored between the two populations (Perez et al. 1999). We chose to associate one CD65 band with group 6 because this produced non-recombinant synteny groups. Placement of this CD65 band in synteny group 7 would have produced a double recombinant synteny group in one individual, which is less likely. The other band was then placed into synteny group 7 because its segregation pattern in the BC_2 was the same at TG438.

Analysis of synteny groups

Given the small number of individuals in the BC_2 population, a process of elimination was used to correlate markers and syntenic groups with PLRV resistance phenotypes. For this analysis, potato clones were classified as susceptible or resistant to PLRV (Table 1) on the basis of field and/or grafting evaluations. This classification of clones was made amenable by the strong and statistically significant expression of PLRV resistance, even in the BC_3 generation. *S. etuberosum* specific markers that were found in either PLRV resistant individuals and lacking in the PLRV susceptible were identified as putative regions associated with PLRV resistance. We accepted markers present in either of the two PLRV resistant clones because at this point is was unclear based on field trials if both clones had the same level of resistance, which would indicate different mechanisms



◀ **Fig. 2** Ideogram of probable order of RFLP and SSR markers in the *S. etuberosum* genome and representation of sytneny groups found in the BC_2 population. Non-recombinant synteny groups 1, 3, 8, 9 and 10 were found but are not represented on the figure. Information used to determine the synteny group order is in the text. The bars represent chromosomal regions for which we have markers unique to *S. etuberosum.* It should *not* be inferred that the markers cover the entire chromosome, nor can genetic distances be calculated. The white bars are absent markers and black bars present markers in an individual. RFLP markers in **bold** text were also used in the E-genome linkage map (Perez et al. 1999). Markers located on different E-genome linkage groups that were attributed to the same potato and tomato linkage group are indicated by arrows. Roman numerals in parenthesis are chromosomal location of a marker in the E-genome map when this location conflicts with its location in A-genome map. The A-genome location was used for synteny group construction because this gave fewer recombinant synteny groups which was considered to be the most likely scenario

of resistance were present in each clone. Using this deductive reasoning, synteny groups 3, 4, and 6 were identified as putative regions associated with PLRV resistance in the BC_2. Subsequent RFLP analysis of the segregating BC_3 population indicated that only markers from synteny group 4 were associated with field resistance to PLRV. Subsequent evaluation of a larger BC_3 population comprising 34 individuals also has confirmed the association of synteny group 4 with PLRV resistance (Kelley and Novy, personal communication).

Discussion

Association of markers with disease resistance

Molecular analysis of the PLRV resistant BC_2 clones, Etb 6-21-3 and Etb 5-31-2, indicated resistance genes may be associated with either TG135 from synteny group 3, or markers from synteny groups 4 (Table 3). Analysis of the BC_3 family, ETB12, with all RFLP markers from synteny groups 3, 4, and 6, and selected markers from the remaining synteny groups, showed that only a *S. etuberosum* specific RFLP marker derived from probe TG443 (DNA digested with *EcoRV* and a band of approximately 6.5 kb scored) from synteny group 4 was present in PLRV resistant

Table 3 Categorization of *S. etuberosum* synteny groups found in the BC_2 individuals as either recombinant or non-recombinant when compared to the synteny groups found in the *S. etuberosum* parent

BC_2	PLRV reaction[a]	Recombinant[b]	Synteny groups	
			Non-recombinant	Missing
5-31-2	R	2, 3, 7, 11[c]	4, 8, 9, 12	1,5,6,10,11
5-31-3	MS	2, 7, 11	5, 8, 10, 12	1,3,4,6,9
5-31-4	S	1, 2, 3,7, 9	5	4,6,8,10,11,12
6-21-3	R	1, 7, 10	3, 4, 5, 6, 9	2,8,11,12
6-21-5	MS	1, 7, 8	5, 10	2,3,4,6,9,11,12
6-21-12	S	2, 3, 7, 11	1, 8, 9	4,5,6,10,12

[a] R = Resistant, MS = Moderate Susceptibility, and S = Susceptible

[b] A synteny group was considered recombinant if one or more markers from that group in the *S. etuberosum* parent were missing in an individual progeny. It is assumed that if all markers associated with a synteny group are present in an individual that this is not because of the fortuitous presence of two chromosomes containing these markers

[c] Synteny group 11 in 5-31-2 consists of only SSR67. SSR67 primers detected two products in *S. etuberosum* of which only one was polymorphic. This may not be the correct synteny group for this marker

Table 4 Results of SSR optimization

SSR name	Annealing (°C)	Expected size in tomato (bp)	Main PCR products from *S. etuberosum* (bp
SSR22	64	217	200
SSR31	64	103	106
SSR67	54	100	61, 144
SSR76	62	199	197
SSR111	62	188	194
SSR128	60	123	92, 122, 136
SSR136	58	148	148
SSR146	62	243	217
SSR188	62	130	148
SSR310	62	148	131
SSR350	58	267	249

SSRs that amplified products near the expected size which uniquely identified *S. etuberosum* are presented. Size of the main PCR product from *S. etuberosum* is an estimation based on analyzing the fragment on the LICOR DNA sequencer or point-to-point fit using Alpha ease software (Alpha Innotec)

ETB12-2, ETB12-3, BC_1 parent P2-3, BC_2 parent 6-21-3 and the PLRV resistant BC_1 control P2-4. TG443 was missing in all PLRV susceptible BC_2 and BC_3 clones. Synteny group 4 is represented by three different 'fragments' in the BC_3 (Fig. 3) which suggests that resistant genes are located close to TG443. Based on this finding, further analyses with RFLP and PCR-based molecular markers closely linked to TG443 in the tomato and potato linkage maps are being conducted in larger populations to identify those closely linked to PLRV resistance.

Significant differences in viral accumulation were found between the BC_3 sibs ETB12-2 and 12-3 even though both were classified as highly resistant based on field trials (Novy and Gillen, unpublished data). The only marker associated with ETB12-3 (lower viral accumulation) and not ETB12-2 (higher viral accumulation), and its susceptible full sibs is TG8 found on LG 9. Marker TG8 is present in the PLRV resistant Etb 6-21-3 and Etb 5-31-2, but also in the field susceptible Etb 6-21-12. Since viral accumulation in Etb 6-21-12 was not tested it could be possible that it does have reduced PLRV accumulation, but lacks the PLRV infection resistance that confers a higher level of field resistance. A larger BC_3 population derived from Etb 6-21-3 and currently being characterized may help in elucidating if PLRV resistance derived from *S. etuberosum* is a combination of resistance to infection and accumulation.

RFLP markers TG22, TG123, TG208 associated with *S. etuberosum* synteny group 4 indicated there may be two copies of a portion of this region, possibly on different chromosomes, or that there may be duplications in the region. There is also evidence that the resistant BC_3 clones may have more copies of portions of this region than the susceptible BC_3 clones. Given that the linkage map of *S. etuberosum* is fragmentary and that we were not able to construct a genetic linkage map but utilized a priori constructed synteny groups in this analysis, it is

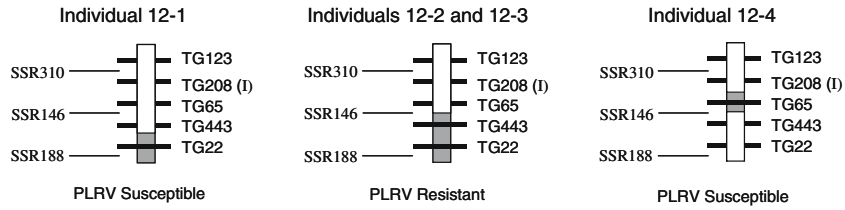


Fig. 3 Ideogram of synteny group 4 in the ETB12 BC_3 individuals. The SSR markers were not screened on the ETB12 BC_3 population, therefore they are noted outside of the ideogram of the synteny group. See Fig. 2 for further explanation of notation

possible that the synteny group 4 markers are detecting two different chromosomes.

A QTL analysis for resistance to PLRV accumulation in a diploid potato population which contained *S. chacoense*, *S. yungasense* and *S. tuberosum* in its pedigree (Marczewski et al. 2001) found a major QTL (PLRV.1) which explained 60% of the variance on linkage group 11 in a cluster of genes with sequence similarity to the tobacco N gene for resistance to TMV. Other QTLs were found on linkage groups 5 and 6. Another QTL for resistance to PLRV accumulation (PLRV.4) was also found in the central region of linkage group 11, which is clearly different from PLRV.1 (Marczewski et al. 2004). This indicates that resistance from *S. etuberosum* may be a unique locus. The BC_2 individuals Etb 6-21-3 and Etb 5-31-2 are resistant to PLRV viral accumulation, but 6-21-3 does not contain any markers from synteny group 11 and Etb 5-31-2 has only one SSR from synteny group 11 which marker's linkage group association is doubtful.

Analysis of synteny groups

Our analysis of the synteny groups in the BC_2 provides evidence of recombination among the A-and E-genomes (Fig. 2). A synteny group was considered recombinant if one or more markers from that group in the *S. etuberosum* parent were missing in an individual progeny. It is assumed that if all markers associated with a synteny group are present in an individual that this is not because of the fortuitous presence of two chromosomes containing these markers; a valid assumption in that the BC_1 generally showed 11-12 (base set) of *S. etuberosum* chromosomes. However, it is possible that the groupings of the markers which we assumed to exist based on the E-genome map and the A-genome linkage maps of potato and tomato may be incorrect. For example, translocations among E-chromosomes relative to published tomato and potato maps could exist. In this case what we believe is recombination among the two genomes may be segregation within the E-genome or even chromosome breakage. Analysis of the RFLP and SSR data showed that 22 of 41 (54%) synteny groups in the BC_2 population were recombinant relative to the 16-1 parent. Recombinant synteny groups were found in the BC_2 for groups 1, 2, 3, 7, 8, 9, 10 and 11. McGrath et al. (1996) analyzed a [*S. palustre* (syn. *S. brevidens*) × *S. tuberosum*] BC_2 population consisting of 76 individuals with RAPDs and RFLPs and found examples of potential recombinants for all E-genome chromosomes except chromosome 5 (McGrath et al. 1996). This study also found that synteny group 5 was not recombinant in the BC_2 and was not transmitted to the BC_3 family. The BC_3 population was analyzed with all RFLPs from synteny groups 3, 4, 6 and 11 and selected probes from the other synteny groups. Therefore, estimates of recombination are not directly comparable to the BC_2. There was no evidence for recombination of synteny groups 6 and 7 in the BC_3. There was evidence for recombination of synteny groups 3, 4 and 9 in the BC_3. McGrath et al. (1994) and Williams et al. (1990) used information and markers from Bonierbale et al. (1988) and Tanksley et al. (1992) to determine marker order. These sources, as well as Perez et al. (1999) and Van der Hoven et al. (2001) were also used in our analysis.

The RFLP markers in common between the E-genome map (Perez et al. 1999) and this work are denoted in bold on Fig. 2. The E-genome linkage groups presented in Perez et al. (1999) consist of 19 linkage groups which are genetically unlinked

at LOD 5, but were presented as aligned with their homologous regions in the A- and tomato genomes. Therefore, the markers in boldface that are shown on the same synteny group yet were genetically unlinked in the E-genome map are indicated with arrows. Analysis of the BC_2 population showed that eight synteny groups appear to be recombinant (Fig. 2) and in groups 1, 2, 3 and 11 the presumed recombination event occurred between RFLP markers that are on different E-genome sub-groups. This would be consistent with a large genetic distance between these groups which would explain why Perez et al. were unable to link them. Conversely, markers from synteny groups 4, 6 and 12 that were on different E-genome sub-linkage groups in the Perez study were only found on non-recombinant synteny group in the BC_2. However, in the BC_3 family ETB12, synteny group 4 was fragmented (Fig. 3). Therefore, the constitution of our synteny groups must be considered as a likely arrangement, not the actual arrangement, of these markers in the E-genome.

Synteny group construction though potentially informative for marker development, sometimes gave contradictory results when used to evaluate the potential for recombination between the genomes. Perez et al. (1999) found through a linkage mapping study that E-genome linkage groups 3 and 7 are homosequential with the A-genome, except for a small putative inversion in the E-genome and would be expected to recombine with the A-genome. These conclusions are supported by our finding of recombinants of synteny groups 3 and 7. Our group 3 had three different constitutions (Fig. 2) in the BC_2 which seems likely if recombination was the cause. Perez et al. (1999) found that E-genome linkage groups 9 and 10 have the same markers as the A-genome but a different order indicating inversions and/or transpositions have occurred. This could be expected to inhibit recombination yet our synteny groups 9 and 10 are recombinant despite the expected differences between A- and E-genomes.

The discovery of markers from all 12 potato chromosomes contradicts the genomic in situ hybridization analysis of clone P2-3, the BC_1 parent, which showed only 11 of the 12 *S. etuberosum* chromosomes were present (Dong et al. 1999). This could be the result of the limitations of the GISH technique. GISH showed that P2-3 had one excess A-genome chromosome and one less E-genome chromosome than expectations, therefore it is possible that the GISH misidentified one chromosome. Conversely, if the lost *S. etuberosum* chromosome had an interchromosomal translocation relative to the A-genome, then molecular analyses might show representation of markers from all 12 A-genome chromosomes.

Progress is being made to introgress the unique PLRV resistance genes from *S. etuberosum* into cultivated potato. On the basis of the expression of a high level of PLRV resistance in three generations of progeny derived from a *S. etuberosum* somatic hybrid, resistance is highly heritable and is likely monogenic or oligogenic. Molecular characterization has localized resistance to chromosome 4 near RFLP marker TG443. There is some evidence that could indicate that resistant clones have two copies of a portion of this region and the susceptible have one or none. Synteny group analysis was not a very effective tool to gain information on genome structure as it gave results that seem to contradict mapping information and it is based on assumptions of marker order that may not be valid. However, it did give an indication that recombination among the A- and E-genomes may be occurring. Additional marker saturation of the region surrounding TG443 is ongoing in a larger BC_3 population in order to identify markers useful in marker-assisted selection for PLRV resistance derived from *S. etuberosum*.

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